Microarray Fabrication Techniques

INVENTOR

Shiping Chen
13818 Goosefoot Terrace
Rockville, MD 20850
Email: chen.shiping@iname.com
Phone: (301) 405-5206 (work)
(301)309-6818 (home)

SPECIFICATION

1. INTRODUCTION

The microarray refers to an array of known biological or chemical samples (termed "probes") immobilized as microscopic spots at predefined positions on a supporting substrate. The array is then flooded with another biological or chemical sample (termed "target"), which interacts with one or multiple probes on the array in a parallel fashion. In DNA microarrays in particular, the probes are oligonucleotide or cDNA strains and the target will be a fluorescence or radioactive labeled DNA sample. The molecular strands in the target will bind with the complimentary strands in the probe array in a process called "hybridization". Finally, the hybridized microarray is inspected by a microarray reader, which uses laser or other energy sources to excite the label and register the DNA composition of the target according to the position and strength of the label emission in the array.

The microarray technology provides an extremely useful tool to conduct biological or chemical experiments in a massive parallel fashion. It is particularly powerful in screening, profiling and identification of DNA samples. The invention described in this disclosure is relevant to all biological or chemical microarrays, including DNA, protein, chemical compound microarrays. For the convenience of description, we will use DNA microarray as an example.

The importance of the microarray technology can never be overstated, as it is based on the fundamental and revolutionary nature of this tool. Its impact on the biotech industry and to the entire health care sector will be felt for the decades to come. The effects will be so fundamental that it can only be paralleled to what the integrated circuits have done to electronic industry and to the Information Age as a whole. First of all, microarray is capable of dramatically boosting the efficiency of traditional biochemical experiments. Tests that would have taken years can now be completed in hours or even minutes. Secondly, the applications of this technology reach almost every corner of the healthcare sector and beyond. These include gene profiling, disease diagnostics, drug discovery, forensics, agronomics, biowarfare and even biocomputers!

All microarrays today come as two-dimensional probe matrixes fabricated on solid glass or nylon substrates. Because the target samples are generally hard to produce or very expensive, it is highly desirable to perform as many binding experiments as possible on a single array. This calls for a significant increase of probe density and quantity on a single substrate. In general, microarrays with probe pitch smaller than 500μm (i.e. density larger than 400 probes per sqr. centimeter) is referred as high density arrays, otherwise, they are terms as "low density" arrays.

There are three microarray fabrication techniques on the market, namely, photolithograph, inkjet and robotic spotting techniques. The photolithograph technique [US Patents 5445934, 5744305] adapts the same fabrication process for electronic integrated circuits to synthesis probes *in situ* mer by mer. The technique requires a huge initial equipment cost running up to hundreds of millions of dollars. The initial setup of new array designs is also very expensive due to the high cost of photo masks. This technique is therefore only viable in mass production of standard microarrays at a very high volume. Even at high volume, the complexity in synthesis still limits the production throughput resulting in a high array cost. For example, a 10,000 probe GeneChip™ from Affymatrix produced in this technique can cost as much as $5,000. This complexity also limits the length of the synthesized DNA strain to the level of an oligonucleotide (~25 bases), which reduces specificity of hybridization in some applications.

The established robotic spotting technique [US Patent 5807522] uses a specially designed mechanical robot, which produces a probe in the microarray by dipping a pin head into a fluid containing an offline synthesized DNA and then spotting the it on to the slide at a pre-determined position. Washing and drying cycles have to be executed before moving on to produce a different probe in the array. Because a microarray contains a huge number of different probes, this technique, although highly flexible, is inherently very slow. Even though the speed can be enhanced by employing multiple pin-heads and spotting multiple slides before washing, the production throughput is still extremely low. For example, it currently takes 7 hours to produce 100 5,000-probe arrays using a commercial spotting arrayer. This

technique is therefore not suitable for high volume mass production of microarrays. In the due-diligent process of this invention, we discovered an emerging new spotting technique, which uses capillaries instead of pins to spot DNA probes onto the support. The new spotting method shares several features of our design but differs in a crucial technical aspect. We shall discuss the difference in detail after the description of our invention. In short, due to this crucial difference, the existing capillary based spotting techniques still cannot realize high throughput fabrication of high density microarrays.

The third arraying technique, which is still under development, employs inkjet technology to deposit individual nucleotides at suitable positions on a supporting substrate and produce an oligonucleotide array through *in situ* synthesis. Currently, no arrayer based on this technology is commercially available. Rosetta is using this method for in house research and Agilent has announced a plan to develop systems based on this technique. We believe that inkjet technique can achieve medium flexibility and medium production throughput, a compromise between photolithegraph and robotic spotting techniques. As a result, it will not offer a significant cost reduction in microarrays.

In summary, at a current price tag of several thousand dollars per 10,000 probes, the microarrays fabricated with existing technologies are far too expensive as a single use lab supply. This prohibitive cost is the major limit in the market penetration of microarray technology.

This document describes the invention of a new microarray fabrication technology termed "gene-stamp" capable of eliminating the problems described above.

2. DESCRIPTION OF THE TECHNOLOGY

In the description below, we shall use DNA microarray as an embodiment of the invention. The technique can also be used to produce microarrays of other biological and chemical materials such as proteins, drug compounds etc.

A microarray fabrication system based on this invention is illustrated schematically in Figure 1. The heart of the system is a print-head comprising a large number of flexible capillaries. Each capillary in the print-head is fluidly linked to a reservoir containing a specific DNA sample. The reservoirs may take the form of fluid wells in standard microtiter plates. Probes are delivered to the print-head via the capillaries and the entire set of probes can be deposited on to the substrate in a single printing action. There is an inspection system to inspect the quality of the fabricated microarrays online or offline.

The basic elements of this technology include methods and apparatus for print-head, fluid delivery, probe deposition and inspection. We will discuss the details of these technological elements in the following sections.

2.1 Print-Head

The print-head gathers probe fluids from their individual reservoirs and deposits them in small volumes on to the microarray substrate at each printing action. A print-head is a solidified piece and can be polished to a high degree of flatness to facilitate uniformed probe deposition onto flat microarray substrates. The print-head may contain a single or multiple capillary bundles. In the multiple bundle configuration, shown in Figure 2, it is preferred that the outline shape of each bundle is rectangular or square so that the capillary bundles can easily be assembled to form a structured matrix in a rectangular print-head. In this way, 1) we can make the best use of the surface area of a standard microscope slide; 2) the position and orientation of each bundle is known to the system; 3) it easier for the system to identify each capillary in a bundle. Capillaries used in the system can be made of silica or other suitable materials such as glass, polymer or metal.

This invention envisages two different configurations for the capillary bundles. These are assembled and unitary bundles.

2.1.1 Assembled Capillary Bundle

This type of capillary bundle is assembled from a large number of individual, ready-made capillaries. Capillaries are bundled together and solidified at the proximal end and eventually assembled into the print-head and left loose at the distal end. Each capillary can be fluidly linked to a probe reservoir, which may be the well in a standard microtiter plate. The linkage can be made permanent by gluing the capillary to a hole at the bottom of a microplate well. Alternatively, as shown in Figure 3, the capillaries can be permanently fixed to a frame, which holds the positions of capillary tips in a grid, which has the same spatial pattern and pitch as a microplate. Then the frame can be locked on to a standard microplate to establish the fluid linkage for each capillary. In this way, the microplate after array fabrication can be taken off the arrayer for long-term storage. It is also possible to wash the capillaries after the fabrication of a particular microarray, then install a new set of microplates to make a different microarray.

This invention discloses two different methods in making the assembled capillary bundle. These are termed as "tight-pack" and "guide-plate" method, respectively.

2.1.1.1 Tight-Pack Method

In the tight-pack method, a large number of hair-thin, flexible capillaries are tightly packed in random order into a bundle at the proximal end, in which, the outer surface of a capillary is in direct contact with that of adjacent capillaries. However, although such a bundle may be used to print a probe array at the highest density, the array is useless because the association between a capillary facet in the bundle and the fluid reservoir that it linked to, thus the probe identity, is lost. One of the key claims of this invention is the CONCEPT that a randomly packed capillary bundle can be made suitable for microarray printing by re-establishing the one-to-one association of each capillary between the proximal and distal end of the bundle AFTER the bundle has been made.

There are a number of ways to re-establish the capillary association in a tightly packed bundle. These are:

1) Use a special type of capillaries that is not only capable of transporting fluid material like a normal capillary but also, transmitting light just like an optical fiber. We can then re-establish the capillary-reservoir association by launching light into each capillary from the reservoir end and observe the position of the exiting light at the bundle end, using a imaging device, as shown in Figure 4. This imaging device can be either a CCD based digital microscope or a scanning microscope. Such special light guiding capillaries can be produced by creating an inner region in the capillary, in which the optical refractive index is higher than the outer region around it. Such a region will be able to trap the light inside it and guide the light all the way through the capillary.

   This light trapping region inside capillary can be created in many different ways. The first method is to coat a normal silica capillary with a polymer with lower refractive index. The second method is to fill normal silica capillaries using a special transparent fluid with a higher refractive index to create a temporary fluid light trapping core. The third, also preferred, method is to draw the capillary out of a special preform. Such a preform can be made by following the modified chemical vapor deposition (MCVD) procedure widely used in the optical fiber industry for optical fiber perform fabrication, but without collapsing the central cavity at the final step. Alternatively, this special preform can also be made by drilling a hole of suitable size at the center of a normal multimode optical fiber preform or depositing a layer of Fluoride doped silica outside a suitable pure silica tube. Since the Fluoride doping lowers the refractive index of pure silica, it forms a cladding to help trapping light inside the pure silica region around the central cavity.

2) Blow air into the capillaries one by one from the distal end and use a micro-flow detection device at the bundle proximal end to locate the outlet of the air flow. The position coordinate of the capillary facet is registered among other capillaries in the bundle. A micro sized hot wire or temperature probe can be used for the flow detection because air current can alter the thermal balance at the probe.

3) Fill capillaries with ink from the distal end and observe the appearance of the ink at the bundle facet at proximal end using an imaging microscope and register its position. Capillaries can be filled one at a time or several at a time using ink of different colors.

4) Use metal capillaries or coat dielectric capillaries with metal. An additional dielectric overcoat is needed to insulate capillaries from each other. The capillary-reservoir associate can be established by verification of electric connectivity between the proximal and distal ends of the capillary bundle.

In order for the system to automatically register the identity of a specific capillary in the bundle established using any of the four methods described above, we have to establish a positional reference that is easily recognizable by the system. This invention provides two different approaches:

1) Absolute coordinates A XY coordinate system can be established with reference to the edges of the bundle and the identity of each capillary can be registered by the system through its unique coordinates. This approach is relatively easy to implement if the outline shape of the bundle is square or rectangular and the capillaries are packed really tight, so that, as shown in Figure 5, they naturally fall into a honeycomb pattern. This method should be able to tolerate a moderate degree of positional randomness in the bundle and therefore should work for most situations.

2) Image matching When the capillaries are completely random and there is not obvious spatial pattern in the bundle, we can employ an image matching method to register capillary identities. In this method, as illustrated in Figure 4, the computer records an image file of the bundle facet in which each capillary is tagged with the ID of the associated fluid well. The spot pattern of the microarray will be a precise hard copy of the capillary facets in the bundle. After hybridization, the microarray is scanned by an array scanner, which generates a pair of digital images at different fluorescent wavelengthes. The scanned image can then be compared to the ID tagged bundle image stored in the computer to establish the DNA identity of each spot in the array. To make the image comparison easier, selected small number of wells in the plate can be filled with a special paint or ink or a fluid tagged with a distinctive dye. These distinctive spots on the scanned image of the probe array can then be used as reference points to match the scanned images with the ID tagged image file of the capillary bundle, pre-stored in the computer.

A bundle consisting of 100,000 or more capillaries can be fabricated and ID tagged in this way. However, it may be more beneficial to limit the number of capillaries in a random bundle to a smaller number, say 1538. Then assemble multiple such random bundles into an orderly bundle matrix as shown in Figure 2 to form a print-head. This is because, firstly, microtitre plates with 1538 or fewer wells are widely in use. Secondly, this arrangement gives us the flexibility to organize gene probes into different groups with one bundle per group, then mix-and-match different groups to produce different arrays for different applications. Finally, this arrangement give the user the option and flexibility to scan only one group of probes on the array, wherever necessary to save time.

Considering a particular embodiment of the above described arrangement:

Presuming capillaries with an outer diameter of 100μm are used and each bundle is linked to a 1536-well or four 384-well microtitre plates, the capillary bundle would have a 4mm x 4mm cross section. 75 such bundles can be easily assembled into a 5x15 orderly bundle matrix, which could produce a microarray consisting 115,200 probes in one stamp and covering a 2cm x 6cm area on a microscope slide.

2.1.1.2 Guide-Plate Method

The guide-plate method for capillary bundle fabrication is illustrated in Figure 6. A guide-plate with an orderly matrix of small holes is first fabricated through precision drilling or other methods (6a). The plate can be made of any suitable material such as metal, glass or plastic and can also be relatively thin and/or deformable and/or fragile. The hole diameter should be slightly larger than the outer diameter of the capillaries to be used. Capillaries are carefully plugged into the holes to form a loose bundle (6b). The

bundle is solidified at the section near the guide-plate using epoxy, cement or other suitable solidification techniques (6c). Finally, the solidified portion is cut at a position very close to the guide-plate (6d).

Because the holes are positioned in an orderly matrix at the guide-plate and the bundle is cut very close to the guide-plate. The spatial position of each capillary in the fabricated bundle will be in an orderly matrix the same as the holes in the guide-plate. On the other hand, because the bundle is in one solid piece, it can be polished to achieve a high degree of flatness and at the same time, is mechanically robust for printing. In addition, since the capillaries are in an orderly matrix, its identities can be established direct through its position in the matrix. No ID tagging procedure is required.

2.1.2 Unitary Capillary Bundle

The assembled capillary bundle described above can be fabricated at a relatively low cost. However, the probe pitch produced by such a bundle is limited to around 50μm because it becomes difficult to control the diameter of the capillary on a draw tower when the outer diameter of a capillary falls below 50μm. This invention provides a unitary capillary bundle in order to further enhance the probe density beyond 50μm pitch.

To fabricate a unitary capillary bundle, rigid glass tube preforms are stacked into an orderly matrix as shown in Figure 7a. The matrix could be in honeycomb or chessboard pattern depending on the outline shape of the glass tube and stacking method. The preform stack is then heated to weld them together. Finally, the welded stack is drawn on a fiber draw tower (Figure 7b). In this process, a section of the tube stack at each end will remain unchanged while the middle section is thinned into a flexible capillary bundle. Each stack can therefore produce two special bundles, one end of which is a 2D array of rigid and large tubes with sizes compatible to the microtiter plates, while the other end is a 2D array of fine capillaries of the same configuration. The positions of each capillary in the bundle are highly organized and can be registered according their position orders in the bundle. Such a bundle is named "unitary" capillary bundle because the capillary and fluid reservoir is a unitary body.

Presuming the original tube preforms have a 3mm x 3mm square shaped cross-section, a 50x50 tube stack would be measured 15cm x 15cm at cross section. If each tube is drawn into a capillary with 20μm outer diameter, the bundle at thin end would measure 1mm x 1mm, and would be sufficiently flexible. The probe pitch produced by this bundle would be 20μm. As a result, the probe density can increase by 25 fold in comparison with the random bundle example described above. In addition, multiple such unitary capillary bundles can be assembled together in an orderly way to produce a stamp head containing an orderly 2D matrix of almost 2 million capillaries.

2.2 Fluid Delivery

The functions of the fluid delivery sub-system in the arrayer are to

- Transport probe fluid from the reservoir to the print-head through its respective capillary;
- Ensure the flow rate to be constant in each capillary and uniform across the print-head.

We shall discuss the implementation of these two functionality in details.

2.2.1 Fluid Transport

This invention offers several methods to drive the probe fluid from its reservoir into the capillary and towards the print-head. They can be used alone or in combination in the fluid delivery sub-system. These methods include:

- Air pressure A differential air pressure can be established and maintained between the proximal and distal ends of the capillary bundles, which will translate into hydraulic pressure to drive the probe fluids.

- Gravity Once the capillaries are filled with the probe fluids, a constant flow can be maintained and controlled by adjusting the vertical positions of the fluid reservoirs, e.g. the microtiter plates, with respect to the position of the print-head.
- Electric field Because DNA fluids are negatively charged, a voltage applied between the reservoir and the print-head can be used to control the flow of the fluid through electrostatic and electroosmotic force (EOF) [1].

2.2.2 Flow Rate Control

In order to ensure that the spot sizes on the substrate are constant from array to array and uniform across each microarray, the flow rate has to be controlled to be constant in each capillary and uniform across the print-head.

To hold the fluid flow in a capillary to a constant rate is relatively easy. All fluid driving methods described in Section 2.2.1 can be used to control the flow rate. However, air pressure and gravity are relatively blunt mechanisms for flow rate control. When air pressure or elevation differences disappear, the flow does not stop instantly due to back-pressure built up in the capillary. In comparison, electric fields is much more precise and responsive in controlling the flow.

The task of ensuring the uniformity of flow rates in every capillaries of the print-head is much harder because the flow rate in a capillary is dependent up on many other factors besides the driving force, which include cavity size and surface characteristic of the capillary and fluid viscosity. Clogging and bubble entrapment in capillaries will also cause missing probes on the fabricated microarray, thus have to be prevented.

This invention provides the following measures to ensure the flow rate uniformity:

- Use of silica based capillaries Silica capillaries are renowned for its precision. Both inner and outer diameters can vary less than 2% in a same draw and less than 5% between different draws. We can therefore use only capillaries made of the same draw to enhance the uniformity. Because the drawing is carried out at melting point of the silica, the surface is extremely smooth by nature. In addition, the silica surface in the capillary is naturally negatively charged, which makes it "phobic" to DNA samples, resulting in minimum friction between sample and capillaries, thus ensures smooth delivery of sample fluids to the print-head. Coating cavity walls with other hydrophobic films such as fluorocarbon polymer may further enhance the durability and uniformity of the capillaries.
- Buffering the probe fluids The viscosities of different probe fluids can be compensated by adding a suitable amount of inert buffering material, such as sugar, to probe fluids.
- Clogging and bubble prevention All probe fluids can be purified and handled in clean room environment to prevent capillary clogging. Fluids can also be preprocessed with ultrasound and vacuum suction to eliminate bubble entrapment.
- Control flow rate in each capillary with individual electric fields With the first three measures described above, it is highly probable that the flow rate variation across the print-head can be kept within a small range (e.g. 20%) under a uniform driving force such as air pressure or gravity. This is sufficient for most of the microarrays. For applications that requires even better flow rate control, electric field method can be used to control the flow rate in each capillary individually. In one specific embodiment of the flow control sub-system, as shown in Figure 8, gravity and/or air pressure have been used as the primary fluid driving force and the electric field is used as an additional, fine adjustment mechanism. The end-facet of the print-head at the proximal end and each capillary tip at distal end are coated with metal. All capillaries are held at a common ground at the print-head and different voltages are applied to the different capillary tips at the distal end. This produces appropriate electric fields to fine-tune the flow rate in the capillary. Because the electric field is only a fine-tuning device, a relatively small voltage is sufficient.

2.3 Probe Deposition

The probe deposition sub-system in the arrayer ensures a constant, uniform volume of probe fluids are deposited onto the substrate and there are no missing or overlapped spots on the microarray. This invention includes two deposition mechanisms: mechanical tapping and electrostatic printing.

2.3.1 Mechanical Tapping

As illustrated in Figure 9, probes can be deposited on to the microarray substrate by mechanically tapping the print-head on the substrate. The constant flow in the capillary produces a micro sphere of fluid at the facet of each capillary (9a). When the print-head is tapped on the substrate, the droplet bonds to the substrate due to surface tension (9b). This surface tension overcomes the binding force in the fluid. The droplet thus breaks away from the fluid column at its weakest point, i.e. exiting point of the capillary cavity, when the print-head withdraws (9c). A probe is deposited on the substrate.

This invention provide the following measures to reduce missing or overlapping spots on the fabricated microarray:

1) The surface of print-head facet should be in perfect contact with the substrate during a tap action. When a microscope slide is used as the microarray substrate, the surface of the print-head facet should be polished to a high degree of flatness.
2) One of the contacting parts, i.e. print-head or the substrate, should be rigidly supported while the other fixed on a soft or spring-loaded platform. If these two surfaces are slightly unparallel, the one on soft support will yield to the one on rigid mounting to ensure perfect contact (Figure 10).
3) In order to prevent the deposited droplet from flowing to the area of other probes thus producing overlapping spots, a micro well can be fabricated at the tip of each capillary (as shown in Figure 9), which can accommodate the fluid volume of the droplet on the substrate. The micro wells can be produced one-by-one using a diamond tipped precision drill. If the capillary has a central region doped with Germanium (originally designed for light transmission as described in Section 2.1.1.1), these micro wells can be fabricated in one go by dipping the print-head into an etching fluid such as Fluoride acid because a very small Ge doping can dramatically accelerates the etching rate of the silica.
4) In addition, the deposition process described here can be assisted by treating the substrate surface to become positively charged and/or treating the surface of the print-head facet to become hydrophobic and negatively charged.

2.3.2 Electrostatic printing

As shown in Figure 11, a metal layer can be coated on the facet of the print-head and the microarray substrate is placed on a metal or metal coated support. When a voltage is applied between the stamp head and the support with positive polarity at the support end, The DNA samples in the capillary will be attracted towards the substrate because of their negative charges. If a short pulse of sufficiently high voltage is applied when the stamp head facet is close to the substrate, sample fluids could be directly tore off the fluid column in the capillary and fly towards the substrate. One advantage of this method is that the stamp head does not have to touch the substrate surface. Because fluid naturally takes sphere shape in air, the deposited spot will be in better shape than that form by surface contact. In addition, the stamp head may not have to move at all and no micro well is needed at the capillary tip.

2.4 Array Inspection

The array inspection sub-system monitors the quality of fabricated microarrays. This can be carried out off line or online and in real-time. Arrays with missing and overlapped spots are automatically detected, registered and eventually rejected as defect products. The device may also be used to monitor the spot sizes in real time and feed the information back to the fluid delivery sub-system to control the flow rate in the capillaries. If the spot sizes are uniformly too large or small in the print-head, the system has the option adjust the printing rate accordingly to compensate the spot size change.

This invention offers two different optical designs for the inspection sub-system.

The first design, shown in Figure 12, is based on based on the detection of light scattered by the spots on the microarray. A fabricated microarray is illuminated with light project at a large angle. A digital camera observes the substrate surface from above. Due to their small fluid volume, probe deposited on the substrate will dry very almost instantly and the high salt content in the probe fluid solution will be separated out and becomes light scatters. At areas on the substrate where there is no spots, the light is reflected at the same large angle to the side, thus registering a dark back ground in the camera. At areas where there is a spot, the light is scattered towards the camera, which will register as bright spots.

The second design, shown in Figure 13, is based on the principle of total internal reflection and suitable for the inspection of spots without scatters. A collimated light beam is projected to the bottom of the surface on which the probe array is deposited. The angle of incidence is designed to be slightly larger than the critical angle of total internal reflection at substrate-to-air interface. A digital imaging camera is used to observe the illuminated region above the substrate surface. In the areas of the surface where there are no probes, total internal reflection occurs and little light can be detected by the camera pixel aimed at this location. In the area where there is a probe, however, the presence of the probe destroys the condition of total internal reflection at substrate-air interface. Part of the light beam will be refracted into the space above the substrate surface and captured by the imager. This method can significantly increase the contrast of most transparent objects.

2.5 Spatial Pattern of the Spots on the Microarray Substrate

The spots on all existing microarrays are arranged in the "chessboard" spatial pattern as shown in Figure 14a. The capillaries in a tightly packed bundle are likely to settle into a "honeycomb" spatial pattern as shown in Figure 14b, thus producing honeycomb spot pattern on the microarray substrate. Capillaries in guide-plate assembled bundles or unitary bundles are also likely to be in the honeycomb spatial pattern. For circular shaped spots, honeycomb is the most area efficient pattern. Compared with the "chessboard" pattern, for example, up to 15% more spots can be accommodated within the same area if they are arranged in the honeycomb pattern with the same pitch.

This invention shall claim microarrays with honeycomb patterned or random spot distribution.

2.6 Other Potential Benefits of Light Guiding Capillaries

The fact that in a particular embodiment described above, each capillary is an optical waveguide capable of transmitting light may have other important utilities in the microarray fabrication. For example, the array substrate can be coated with a layer of light sensitive material, which is hydrophobic in dark and becomes hydrophilic after exposure. A light pulse can be sent down the capillary at the very moment that the stamp head touches the substrate. It will make the region immediately under the micro-fluid well at each capillary tip hydrophilic while leave the rest of the substrate surface hydrophobic. In this way, not only the probe will be confined to a well-defined area, target sample fluid will also concentrate in the probe region during hybridization stage, which helps to improve hybridization efficiency and reduce the required amount of target fluid. We may also choose the write substrate coating material and light wavelength, so that the substrate-probe cross-linking can happen instantly when the probe is laid down in fluid phase. It is also possible to incorporate photon cleavable linkers in the probe samples and alter the molecular structures of certain probe or to prevent the fragment from entanglement when they are being laid.

3. Comparison with Prior Arts

The arrayer based on the invention described above is simple and low cost and will be capable of producing one high-density (down to 1μm probe pitch), large scale (500,000 or more probes per slide) microarray in a single stamping action. The production throughput for a single arrayer can be as high as 20 per second. Such a throughput gives it unparalleled advantage in production of high volume, standard microarray

products. In addition, it has great flexibility for custom arrays as the entire or part of the capillaries in the stamp can be quickly wash-cleaned and reused for different probe samples.

In comparison with the lithography based arraying technique, this invention will deliver at least 100 fold reduction in the cost of initial equipment and array set up and at least 10 fold increase in production throughput. Comparing with the pin-spotting techniques, it will have 100-1000 fold enhancement in production throughput. In comparison with ink-jet based arraying technique, the invention should still hold about a 100-fold improvement in terms of production throughput. As a result, this invention can lower the array production cost to below $1 per array, excluding probe production and licensing costs.

Recently, we come across three patent applications, which use capillary based spotting technique for array fabrication. These are

- WO 98/29736, "Multiplexed molecular analysis apparatus and method", by Genometrix Inc., priority date: 12/31/1996
- WO 00/01859, "Gene pen devices for array printing", by Orchid Biocomputer Inc., priority date: 7/2/1998
- WO 00/13796, "Capillary printing system", by Incyte Pharmaceuticals Inc., priority date: 9/9/98

Although all of them use individual capillaries to deliver the probes from a reservoir on to a substrate, we believe all these techniques will have difficulty to produce more than a couple of hundred probes in a single stamp and the minimum probe pitch will be limited to several hundred micrometers, both are significantly worse than what achievable with the invention described here.

Both WO98/29736 and WO00/13796 propose to use a fixture to hold the positions of capillaries near their tips to form an orderly matrix. In WO98/29736, long flexible capillaries are used to deliver probe samples from distant reservoirs; while in WO00/13796, short capillaries are used and the reservoirs are integrated in the fixture. The problem is that it is very difficult to fabrication this fixture with high capillary density using today's mechanical fabrication technology. A key requirement here is to produce holes in very small diameters through relatively thick, hard materials. The material has to be rather thick and hard in order to be reasonably rigid and durable during stamping action. Currently, it is difficult to drill holes with a diameter less than 200μm and a depth/diameter ratio more than 5. This means that it is very challenging to make capillary pitch smaller than 300μm and the thickness of the fixture more than 1mm. This limits the probe density to less than 1000 per sqr. centimeter. In addition, a fixture less than 1mm in thickness and with a large number of holes will easily be deformed out of shape if the dimension is larger than 1cm X 1cm, which means a large-scale microarray consisting tens thousands of probes over an area at least 2cm X 2cm can only be fabricated with several separate stamps. It is the opinion of the author, that the two inventions above, when reduced to practice will only be able to produce several hundred probes in one stamp, which is a improvement over the pin-spotting technique but still far inferior to the technique described in this invention.

WO 00/01859, on the other hand, completely ignored the issue concerning how each capillary should be positioned and identified. In the demonstration given, rigid stainless steel capillaries with outer diameter about 1mm were used. At this small scale and with rigid, large tubes, it is practical to pack the tubes into an orderly matrix, as demonstrated in the application. However, with tens thousands of hair thin capillaries, the issue of individual capillary positioning and identification becomes crucial. WO 00/01859 will not be able to reduce to practice for large scale, high density array fabrication. The practical limit to this technique is again several hundred capillaries with outer diameter probably larger than 300μm.

In summary, we believe the invention proposed here is still the only technology capable of producing a large-scale, high-density microarray with a single stamp action, the true "GeneStamp™".

REFERENCE

Camilleri P, "Capillary Electrophoresis, Theory and Practice", CRC Press, ISBN 0 8493 9127 X